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II. The Composition of the Unsaponifiable Matters and the Purgative Action of the Oils on Mouse

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Summary

1. The composition of the lipid of *Ruvettus pretiosus* was determined by column chromatography with silica gel and was found to be composed mostly of wax and a small amount of glyceride, which are remarkably different from the oil of common edible fish. But the lipids of the testis and viscera are consisted of 28 to 45 per cent of the glyceride and 22 to 41 per cent of the wax in the total lipid.

2. The unsaponifiable matters in the oil were found to be from 91 to 96 per cent of the alcohol, 2 to 5 per cent of the polar lipid and a little hydrocarbon and sterol.

3. The purgative property of the lipid of *Ruvettus pretiosus* for mouse was different from that of common lienteric diarrhea. Its activity was due principally to the high content of wax in the oil which produced a soft stool and discharge of unabsorbed wax which was unmixed with the fecal matter.

In a previous paper (1), the authors have determined the fatty acid composition of the lipid of castor oil fish, *Ruvettus pretiosus*, and reached substantially the same results as the recent works of Nevenzel et al. (5) and Mori et al. (4) in which the existence of hydroxy acid demonstrated by Cox and Reid (1932) (2) was denied. Kimura (1926) (3) and Mori et al. (1966) (4) reported that the fish lipid consisted mostly of cetyl and oleyl alcohols. According to Cox et al. there occurred a small amount of cholesterol and squalene in it (8). They supposed that the purgative property of this oil was due to a large amount of hydroxy acid making up 13 per cent of the total fatty acids. As fore-mentioned, however, there is no hydroxy acid in it. Nevenzel et al. thought that a large amount of wax alcohols was a main cause of the purgative action, though they did not give the certification by animal experiment. Mori et al. found that rats and cats fed with a diet including 7 g of flesh of *Ruvettus pretiosus* per animal per day had diarrhea and supposed that the purgative property was caused by the presence of a fair amount

of alcohols. However, it must be pointed out that "Irikawa" (roasted skin of sperm whale), is often eaten as a side-dish in western Japan, regardless of its having a high content of unsaturated alcohols like lipid of *Ruvettus pretiosus*. Furthermore, we commonly eat a relish of salt dried roe of mullet (*Mugil cephalus*) "Karasumi" in which the lipid consists of 60 to 70 per cent wax esters. (6, 7)

The question is still pending whether the purgative action of *Ruvettus pretiosus* lipid is attributed solely to these unsaturated alcohols. The present paper deals with the experiments conducted by the authors on the quantitative analysis of the constituents of unsaponifiable matters in this fish lipid and on the feeding experiments in relation to its purgative component.

Experiments and Results

Materials

The materials used are the same as those in a previous study.

Isolation of Lipid Classes

The separation of the lipid classes was achieved by Kato's method, (9) using chromatography on a column (70×10 mm) with about 25 g of silica gel (Kanto Kagaku Co. Ltd.) and the sample of about 50 mg was eluted by the developing agents seen in Table 1. The five fractions such as hydrocarbon, wax, glyceride, polar lipid (free alcohol, sterol and fatty acid) and phospholipid, were obtained. Each fraction was analyzed with thin-layer chromatography of the Mangold's method,(10, 11) employing a chromatoplate with silica gel (Wako Gel B-5) activated at 110°C for 2 hours. The composition of the lipids of muscle, bone, testis and entrails (excluding liver and heart) are shown in Table 2. The lipid of muscle and bone contained about 93 per cent wax and about 3 per cent glyceride. 22 per cent

TABLE 1. *Fraction of the Lipid of "Ruvettus pretiosus" by Column Chromatography.*

Solvent	Volume (ml)	Fraction
Petroleum ether	10	Hydrocarbon
Petroleum ether-Ether (99:1)	20	Wax
Petroleum ether-Ether (96:4)	20	Glyceride
Petroleum ether-Ether (90:10)	20	Glyceride
Petroleum ether-Ether (50:50)	20	Alcohol and Sterol
Ether-Methanol (75:25)	20	Fatty acid (with a little alcohol)
Methanol	120	Phospholipid

Column :70×10 mm, silica gel.
Elution by solvent : 1 ml/min.
Petroleum ether :B.P. 60~70°C

TABLE 2. *Composition of the Lipid of "Ruvettus pretiosus"* (%).

Fraction \ Part	Muscle	Bone	Testis	Entrails
Hydrocarbon	trace	trace	trace	trace
Wax	93.28	93.13	22.43	41.50
Triglyceride	3.10	3.31	45.10	27.81
Polar lipid	1.89	1.53	10.72	10.28
Phospholipid	1.55	2.04	21.83	20.39

wax and 45 per cent glyceride were found in the testis and while 40 per cent wax and 28 per cent glyceride were in the entrails. In addition, 10 per cent polar lipid and 20 per cent phospholipid were present in the testis and entrails.

Fraction of Unsaponifiable Matters

The method for fractionation of the unsaponifiable matters was also carried out by column chromatography with silica gel. As shown in Table 3, four fractions were obtained.

Each fraction was determined by thin-layer chromatography. The analysis of sterols, however, was made by the following method, because the separation of alcohol from sterol was unsuccessful owing to the tailing of chromatogram. Firstly the sterol fraction mixed with alcohol was rechromatographed with silica gel after extraction by organic solvent. Then the eluate was refined by the Truswell's method (12) using thin-layer chromatography. The plate was sprayed with $AgNO_3$ reagent, treated by a mixture of chloroform-acetone (98:2 v/v) used as a developing agent, and visualized with a solution of ethanolic-phosphomolybdic acid. We found only one spot of cholesterol almost completely separated from the alcohol on the plate for flesh, bone and skin. Thus, the cholesterol content was determined

TABLE 3. *Composition of the Unsaponifiable Matters of "Ruvettus pretiosus" Oil* (%).

Part of body	Hydrocarbon (Squalene: Pristane)	Alcohol	Sterol	Polar substance
Dorsal part	2.82	91.84	0.65	4.69
Ventral part	1.84 (84.84:15.16)	94.58	0.74	2.84
Tail part	2.35 (87.45:12.55)	94.15	0.71	2.75
Outer part of meat (0~5 mm)	1.84 (87.74:10.26)	95.59	0.42	2.25
Inner part of meat (45~50 mm)	0.52 (85.91:14.09)	93.74	0.80	4.72
Dark meat	2.41 (87.91:12.09)	94.56	0.99	2.04
Skin	1.63 (95.19: 4.81)	94.76	0.85	2.75
Bone	1.16	96.06	1.00	1.87

by the method of Abel using Lieberman-Burchard reagent, (13) after being eluted from the plate. The amount of alcohol was obtained by subtracting the amount of cholesterol from the whole of the sterol-alcohol fraction. The hydrocarbon was separated by gas-liquid chromatography with a polyethylene-glycol-succinate column as described in a previous paper. As shown in Table 3, the alcohol was contained at a level of 92 to 96 per cent in almost all parts except the extrails. In addition, 2 to 4 per cent of polar substance, which was thought to be mostly phospholipid was found. The hydrocarbon consisted principally of squalene in all the tissues tested.

Purgative Action of Oils on Mouse

The oil of *Ruvettus pretiosus* and its fractionated portions were used as the experimental materials for mouse. In addition, sperm whale oil, sesame oil, cotton seed oil, soybean oil and saury oil were also examined. At the same time, we employed other agents such as magnesium sulfate, castor oil, ricinoleic acid, rhuburb extracts, glycerol and sodium alginate for comparison with the purgative property of *Ruvettus pretiosus* lipid. The sample was orally administrated at a dose of 0.1 to 5.0 per cent to the weight of mouse (D.D.), which was about 20 g on an average, with baby's cannula once a day. The animals were fed with pellet prepared as a feed for rat and mouse by the Oriental Kobo Co. Ltd. The characteristics of the oil used are shown in Table 4.

TABLE 4. *Characteristics of the Oil Used in the Oral Administration for Mouse.*

Oil	Acid value	Iodine value	Peroxide value
Ruvettus pretiosus oil	3.31	82.6	1.31
Muscle oil of sperm whale	4.38	83.7	6.28
Blubber oil of sperm whale	2.27	70.3	1.56
Sesame oil	1.07	119.5	6.93
Soybean oil	0.27	140.4	1.87
Cotton seed oil	0.22	121.2	

The results of the feeding experiments are summarized in Table 5.

It was found that the purgative property of the fish lipid for mouse was different from common lienteric diarrhea. In other words, the excrement was not liquid but soft which was composed mostly of undigested food. At the same time, a waxy substance was separately excreted from the bowels and unmixed with the undigested food. We tentatively designated this symptom as a soft and waxy stool. It was noticed that the lower half of the animal body was frequently stained with the waxy substance.

The fish oil was divided into two fractions such as the saponifiable and unsaponifiable matters. Each part was orally administered to the mouse. The

TABLE 5. *Purgative Action of Various Oils on Mouse.*

source \ per cent of dosage to body wt.	5.0	4.5	4.0	3.5	3.0	2.5	2.0	1.5	1.0	0.5	0.4	0.3	0.2	0.1
R. pretiosus oil				+	+	+	+	+	−	−	−	−		
R. pretiosus oil				⊕	⊕	⊕	⊕	+	−	−	−			
(saponifiable matters)														
R. pretiosus oil					+	+	+	+	−	−	−			
(unsaponifiable matters)														
Blubber oil of sperm whale	+			+	+	+	+	+	+	+	−	−		
Muscle oil of sperm whale	⊕			⊕	⊕	⊕	+	+	−	−				
Sesame oil	⊕	⊕	⊕	⊕	⊕	⊕		⊕	+	+	+	+	−	−
Soybean oil	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	⊕	+	+	−	−	
Cotton seed oil	⊕					⊕	⊕	⊕	+	+	+	−	−	
Saury oil	⊕	⊕	⊕	⊕		⊕	⊕	+	+	+	+	−	−	
Magnesium sulfate (20%)									⊕	⊕	+	+	−	−
Caster oil						⊕	⊕	⊕	⊕	⊕	⊕	⊕	−	−
Ricinoleic acid						⊕	⊕	⊕	⊕	⊕	−	−	−	−
Rhuburb extracts (10%)			⊕	⊕	⊕	+	+	+	+	−	−			
Glycerol								+	+	+	−	−		
Sodium alginate (2%)													+	+

⊕ fatty diarrhea
\+ soft stool and waxy discharge
− non-purgative.

saponifiable matters produced fatty diarrhea but the unsaponifiable matters made a soft and waxy stool as in the case of whole oil of *Ruvettus pretiosus*. With increasing amounts of the unsaponifiable matters the evacuation increases. However, the quantity of excrement in mouse decreases when the lipid is given at a level of over 2 per cent per animal weight per day. In this case we observed that the gastrointestinalis of the animal were swollen with food and that the liver was congested.

It is little known that purgation occurs by taking a large amount of common vegetable oil mostly composed of glyceride. But we demonstrated that the experimental animal fed a diet including sesame oil or cotton seed oil at a level of more than 1.5 per cent to its body weight per day caused fatty diarrhea. Saury oil also showed the same symptom. The blubber oil of sperm whale which consists principally of wax caused a rather small soft stool and at the same time the unabsorbed wax was discharged and unmixed with the fecal matter. It was noticed that the gastrointestinalis were expanded with food, as in the case of lipid of castor oil fish. However, the muscle oil of sperm whale which is low in wax content but high in glyceride produces fatty flux.

The common laxatives used as medicine are classified into three groups by the book of "Yakurigaku" editted by Morishima as follows; the first is that rhuburb extracts, castor oil, ricinoleic acid and glycerol stimulate the intestinal canal and activate the movement of bowel; the second is that the inorganic salt such as magnesium sulfate which makes the intestinal canal degenerative and suppresses the absorption of food; the third is that sodium alginate which is indigestive in the tract and causes mechanically excretion. As seen in Table 5, the oil which is high in glyceride content produced fatty diarrhea hindering the intestinal function and often had a lethal effect on the mouse, but the oil which is low in glyceride caused little purgative activity. Comparing castor oil with ricinoleic acid which is a main component of castor oil, the latter was less purgative than the former. This means that the ricinoleic acid is more a stimulus to the intestinal canal than the castor oil and causes the peristalsis. On the other hand, castor oil is partly hydrolyzed in the intestinal canal and the ricinoleic acid thus produced makes the peristalsis for the animal and most of the non-hydrolyzed oil mechanically goes down the intestinal canal, as does common edible oil. A high content of ricinoleic acid had an injurious effect on the mouse and frequently killed it. Sodium alginate which acts as a lubricant in the intestinal canal produces a soft stool which is not fluxed but resembles, in appearance, that of fish lipid.

Considering the fact that the unsaponifiable matters of *Ruvettus pretiosus* consisted mainly of alcohols, with a little squalene, cholesterol and phospholipid, its purgative properties seemed to be responsible for the alcohols, complemented by the role of a small amount of squalene. In fact, the higher alcohols which are composed of the unsaponifiable constitutent at a level of more than 90 per cent of muscle oil and bone oil have been demonstrated as a purgative substance by Cox and Reid, Nevenzel et al. and Mori et al. In addition, we present a new idea that the purgative activity was largely due to the high content of wax in *Ruvettus pretiosus* oil accompanied by a partial role of the higher alcohols. Thus we see that the purgative activity of the unsaponifiable constituent is less than that of the whole lipid of fish which is high in wax content and is principally responsible for the mechanical action as a lubricant but not as a chemical stimulant. Therefore the action resembles, in some sense, that of sodium alginate. In general, the purgative activity of oil is due not only to the wax content but also to the glyceride in the oil. Different symptoms are noticed between a single dose of wax and a single dose of glyceride to the mouse; that is the wax provokes a soft and waxy stool but the glyceride a fatty diarrhea. As the lipid of *Ruvettus pretiosus* consists of wax and glyceride, its purgative property shows both symptoms but is rather characteristic in a soft and waxy stool due to the higher content of wax. Moreover, it is conceivable that the difference between the digestivity rates of both lipids in the intestine or the difference between their lyophilic properties with other fecal matters indicates two kinds of purgative effects.

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